# PHOTOMETRIC DETECTION OF ANTIBIOTIC RESIDUES IN MILK BY A MICROBIOLOGICAL SYSTEM

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## SUMMARY

The aim of this study was to determine the detection capabilities of the ResScreen microbiological system (BT and BS Bioassays) for 26 antimicrobial agents in milk by photometric measurement. In order to carry out this study 16 replicates of twelve concentrations using samples of milk from individual cows were tested. The detection capabilities, determined by means of logistic regression models for the BT and BS bioassays were, respectively (μg/l): amoxicillin (8, 5), ampicillin (8, 4), cloxacillin (42, 42), oxacillin (19, 17), penicillin G (4, 4), cefadroxil (120, 180), cephalexin (96, 150), cefoperazone (80, 120), ceftiofur (75, 90), cefuroxime (113, 140), clortetracycline (400, 3300), oxytetracycline (118, 630), tetracycline (180, 620), sulfadiazine (41200, 106), sulfamethazine (3300, 355), sulfamethoxazol (9800, 78), sulfathiazole (10700, 115), gentamycin (640), neomycin (1200), streptomycin (2600), erythromycin (350), lincomicin (350), tylosin (100), ciprofloxacin (2855), enrofloxacin (2000) and marbofloxacin (3900). The simultaneous use of both bioassays identifies betalactam, tetracycline and sulfamide residues in milk. Also, neomycin, tylosin and lincomycin residues can be detected, but their residues can be confused with betalactam antibiotics since these molecules produce positive results in BT and BS bioassays.

*Key words:* antimicrobial, detection capability, milk, microbial inhibitor test, photometric detection.

## RESUMEN

### Detección fotométrica de residuos de antibióticos en leche mediante un sistema microbiológico.

El objetivo de este trabajo ha sido determinar las capacidades de detección del sistema microbiológico ResScreen (Bioensayos BT y BS) sobre 26 agentes antimicrobianos empleando mediciones fotométricas. Para llevar a cabo este estudio, se ensayaron 16 replicas de doce concentraciones

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utilizando muestras de leche procedentes de vacas individuales. Las capacidades de detección de los bioensayos BT y BS, calculadas mediante el modelo de regresión logística, fueron las siguientes (µg/l): amoxicilina (8, 5), ampicilina (8, 4), cloxacilina (42, 42), oxacilina (19, 17), penicilina G (4, 4), cefadroxilo (120, 180), cefalexina (96, 150), cefoperazone (80, 120) ceftiofur (75, 90), cefuroxime (113, 140), clortetraciclina (400, 3300), oxitetraciclina (118, 630), tetraciclina (180, 620), sulfadiazina (41200, 106), sulfametazina (3300, 355), sulfametoxazol (9800, 78), sulfatiazol (10700, 115), gentamicina (640), neomicina (1200), estreptomicina (2600), eritromicina (250), lincomicina (250), tilosina (100), ciprofloxacina (2855), enrofloxacina (2000) y marbofloxacina (3900). El uso simultáneo de ambos bioensayos permite identificar residuos de antibióticos betalactámicos, tetraciclinas y sulfonamidas en la leche. También pueden detectarse residuos de neomicina, tilosina y lincomicina, pero debido a que estas moléculas producen resultados positivos a los bioensayos BT y BS, sus residuos pueden confundirse con antibióticos betalactámicos.

*Palabras clave:* antimicrobianos, capacidad de detección, leche, método de inhibición microbiológica, detección fotométrica.

## INTRODUCTION

The presence of certain antibiotic residues in milk constitutes a potential risk for the consumer as they may cause allergic reactions as well as interference with intestinal flora and the development of resistance to antibiotics (Dewdney *et al.*, 1991; Currie *et al.*, 1998; Demoly & Romano, 2005; Wilke *et al.*, 2005). From a technological point of view, antibiotic residues can produce important losses in fermented products, such as cheese (Mourot & Loussourorn, 1981; Brady & Katz, 1987; Packham *et al.*, 2001; Berruga *et al.*, 2007; Bradley & Green, 2009). Therefore, monitoring antibiotic residues is very important in controlling food safety. For these reasons, the Codex Alimentarius Comission has established safe levels of antimicrobials in edible tissues (Codex Alimentarius, 2009), and the EU has regulated the Maximum Residue Limits (MRLs) allowed in milk and other animal foodstuff in the Council Regulation 37/2010/CE.

The European Union from EEC657/2002 Directive (Commission Decision, 2002) classifies the analytical methods for detection of inhibitory substances as qualitative or quantitative methods based on their characteristics and methodology. Thus, several commercially available qualitative tests have been developed for the swift and precise detection of the presence of antibiotic residues in milk (Diserens *et al.*, 2005; Toldra & Reig, 2006). Many of the screening tests are based on the inhibition of microorganism growth caused by the presence of drug residues. Among the most widely used microorganisms, *Geobacillus stearothermophilus* subsp. *calidolactis* has been employed in tests such as Delvotest (Kelly, 1982; IDF, 1991), BRT-AiM (IDF, 1991; Müller & Jones, 1993), Eclipse (Montero *et al.*, 2004), Charm AIM-96 (Zomer & Lieu, 1995) and "BT"-"BS" ResScreen (Nagel *et al.*, 2011).

The ResScreen® microbiological system uses two bioassays: "BT" (betalactams and tetracyclines) and "BS" (betalactams and sulfonamides) containing *Geobacillus stearothermophilus* subsp. *calidolactis*